

The University of Chicago

A NEW METHOD FOR THE ASSAY
OF POSTERIOR PITUITARY
EXTRACTS

A PART OF A DISSERTATION SUBMITTED TO
THE FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHARMACOLOGY
1938

By
JULIUS M. COON

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A NEW METHOD FOR THE ASSAY OF POSTERIOR PITUITARY EXTRACTS ⁽¹⁾

BY

JULIUS M. COON

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Of the multiple physiological effects produced by extracts of the posterior lobe several have been applied as the basis for the assay of the extracts, but heretofore no blood vascular response of any type has served as the physiological basis for the determination of the oxytocic principle where the response was proved to be due to the oxytocic principle itself. Blood pressure responses in dogs and cats have been used for quantitative estimation of potency of pituitary extracts since HAMILTON (1) (1912) first developed a method. DI MATTEI (2) suggested a method of standardizing the extracts by their effects in producing cyanosis in the comb of roosters. These were circulatory effects due to the pressor principle.

PATON and WATSON (3) in 1912 discovered that pituitary extracts injected intravenously in the duck caused a precipitous fall in blood pressure accompanied by a splanchnic dilatation as recorded on the plethysmograph. This was confirmed by HOGBEN and SCHLAPP (4) who used depressor free pituitary extracts. They adduced evidence that the depressor response was not due to choline, or to histamine or histamine-like substances since the response was abolished by treatment of the extracts with alkali. HOGBEN (5) testing other tissue extracts clearly proved that the avine depressor response is a specific property of posterior pituitary extracts. This author also showed that the reaction is still obtained after exclusion of the splanchnic circulation. GADDUM (6), working with Kamm's purified pressor and oxytocic fractions, observed that the depressor effect was obtained with much smaller doses of oxytocin than of vasopressin. This was subsequently confirmed by MORASH and GIBBS (7), HOLTZ (8), and DIETEL (9).

In view of the available literature there appeared the possibility that the blood pressure response in the fowl could be used as the basis for the bioassay of the oxytocic content of posterior pituitary extracts. At present the official U.S.P. XI method of measuring the strength of posterior pituitary extracts is that based on the response of the isolated virgin

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guinea pig uterus. This method was originally proposed by DALE and LAIDLAW (10) in 1912. After frequent modifications and improvements the method is now at best successful in the hands of experienced assayists because of the necessity of rigid precautions and difficultly controlled conditions.

Consequently it was thought that it would be a decided advantage if a simpler method could be devised. The present work was undertaken to determine whether the blood pressure response of the domestic fowl could justifiably be used in estimating the oxytocic content of posterior pituitary preparations, and also to determine to what extent the degree of response was influenced by the presence of the pressor hormone or other possible interfering substances.

METHOD

Blood Pressure.—For recording the blood pressure of the chicken the mercury manometer is employed. Due to the rapid heart rate of 300 to 400 per minute, inertia in the mercury manometer must be minimized by using glass tubing of 2.5 to 3 mms. inside diameter. With a fine drawn out glass tube set in a hard rubber float the pulse excursion is usually 1 or 2 mms. high. A filtered 5 per cent solution of U.S.P. XI sodium citrate is used as an anticoagulant. This reagent when injected intravenously into the chicken causes a fall of blood pressure followed by a prompt return to normal, similar to the changes produced by pituitary extracts. On several occasions the injection of 0.1 cc. of the 5 per cent sodium citrate caused about a 10 mm. fall in blood pressure. This anticoagulant, however, does not influence the response to posterior pituitary preparations. With a manometer of 2.5 mm. bore a fall of blood pressure of 40 mms., will force slightly less than 0.1 cc. of fluid from the canula into the artery. A large proportion of this fluid is blood. Furthermore, if the canula is placed properly and the small arterial branches tied off for a distance of about 2 cms. from it, 0.1 cc. of the mixture in the canula can enter the artery without being washed into the general circulation. The citrate solution injected arterially in a dose of 0.1 cc. causes a reduction in blood pressure about half that caused by intravenous injection. If a significant amount of the solution does enter the circulation equal volumes can be assumed to enter with equal pressure changes.

Choice of Anesthetic.—From a series of seventy white leghorn chickens in which the weight, sex, and initial blood pressures were recorded it was found that roosters ranging in weight between 1.8 and 2.2 kgms. and anesthetized with 200 mgms. per kgm. of sodium phenobarbital constituted the most satisfactory assay subjects. Table 1 shows: (1) that all birds under phenobarbital had an average of 17 mms. Hg higher initial blood pressure than all birds under urethane; (2) that all roosters

under phenobarbital averaged 18 mms. Hg higher blood pressure than all hens under phenobarbital; and (3) that all roosters ranging in weight between 1.8 and 2.2 kgms. under phenobarbital averaged 11 mms. higher than roosters of all other weights under the same anesthetic. The dose of urethane used was 1.5 to 2.0 gms. per kgm., that of sodium

TABLE I

Data correlating Sex, Weight, and Anesthetic with blood pressure

B.P. in mms. Hg., Wgt. in Kgms.

Na-phenobarbital						Urethane			
Roosters 1.8-2.2 Kgms.		Roosters < 1.8 Kgms. > 2.2 Kgms.		Hens		Roosters		Hens	
B. P.	Wgt.	B. P.	Wgt.	B. P.	Wgt.	B. P.	Wgt.	B. P.	Wgt.
120	1.8	110	3.3	75	3.5	80	2.5	90	1.8
120	2.0	115	2.6	100	3.0	85	2.7	85	1.4
110	2.2	110	2.4	95	2.0	90	1.5	80	2.4
120	2.1	80	2.5	95	1.5	85	1.7	85	1.9
110	2.0	125	2.4	80	2.5	80	2.0	100	2.1
120	1.9	100	3.0	90	2.6	100	2.1	85	1.8
110	2.0	90	1.5	90	2.1	90	1.9	90	1.6
120	2.0	100	2.8	110	2.2			85	1.5
110	2.1	110	2.6	80	1.9			100	2.0
125	2.2	110	1.6	100	2.1			80	2.0
80	2.0	105	1.5	90	1.8			80	2.2
130	2.1			95	1.8			85	1.5
120	1.9			110	2.3				
110	1.8			90	2.1				
120	2.1			100	2.2				
120	2.1			100	2.4				
120	2.0			95	1.2				
120	2.0			90	2.5				
				100	1.7				
				100	1.9				
				90	1.8				
B. P. Averages	116		105		94		87		87
			112		94				
			104				87		

phenobarbital 180 to 200 mgms. per kgm. The blood pressure was not materially influenced by the dose of either anesthetic within the range given. The advantage of a high blood pressure is clear when one views

Table II which shows the diminishing response to pituitary extract with decreasing pressures. KAUPP (11) recorded an average of 135 mms. of Hg pressure in the femoral artery of 13 cocks using no anesthetic. PATON and WATSON (3) using the duck do not mention an anesthetic. In another paper (12) PATON describes a method of decapitating the duck and states that the blood pressure in such a preparation is usually about 50 mms. Hg. Previous workers (4, 5, 6, 7, 13) have recorded

TABLE II

Data correlating height of blood pressure with sensitivity to the first Injection of 0.02 cc. Pituitary Extract

B. P. in mm. Hg. . . .	120	110	100	90	80
No. of chickens	11	6	11	9	7
Average fall in B. P. . . .	19	13	11	8	5

low blood pressures using urethane supplemented with ether, or high blood pressures with low sensitivity using ether alone. Experiments in this laboratory similarly show that the high blood pressures of ether anesthesia do not, as might be expected, conduce to high sensitivity to pituitary extracts and that in the case of the lower blood pressures under phenobarbital anesthesia the response is significantly greater. The conclusion is reached from this and other considerations that phenobarbital sensitizes the vascular system to the depressor substance of pituitary. MORASH and GIBBS (7) observed that the injection of oxytocin often caused struggling of the bird, an observation made in this laboratory when urethane was used in a dosage of 1.5 gms. per kgm. This did not occur with 2.0 gms. per kgm. of urethane or 200 mgms. per kgm. of sodium phenobarbital. It may be emphasized that the animal must be kept in a steady state of narcosis and free from movements and fluctuations in blood pressure during the assay.

The chicken is surprisingly tolerant toward sodium phenobarbital. The comb color remains a healthy bright red throughout the anesthesia, and rectal temperature is depressed 0.5 to 1.5° C. In an experiment two chickens receiving 200 mgms. per kgm. recovered in 24 to 48 hours. It is known that a dose smaller than this is lethal for cats.

Preparation of the chicken.—For recording blood pressures in the duck and fowl previous workers have canulated the carotid artery, and some have experienced difficulties. GIBBS (13) states that these difficulties largely disappear if the canula is inserted into an artery of the

thigh, which he called the popliteal artery. In this work all blood pressures were recorded from the ischiadic artery.

Two hundred mgms. per kgm. of sodium phenobarbital are injected into the gastrocnemius muscle one to two hours before the operation. When the chicken is deeply anesthetized, it is placed on its right side in a relaxed position with the femur at right angles to the spine and the tibia, and its feet tied to the board. The feathers are pulled from the outer surface of the left thigh. A 7 or 8 cm. incision is made in the skin about 1.5 cms. below and parallel to the femur and extending from a point approximately 2 cms. posterior to the distal end of the femur. This exposes the large gluteus primus muscle. The lower flap of skin is pulled down so that the posterior edge of the gluteus primus is seen lying over the semitendinosus muscle. This edge is separated from the underlying muscle for the length of the incision. When the free edge of the gluteus primus is then lifted up the ischiadic artery, ischiadic nerve, and crural vein may be seen coursing along the anterior edge of the semitendinosus. Anatomical terminology used here is taken from KAUPP (14). To gain easy access to this field the gluteus primus is cut near the proximal end of the incision at right angles to its free edge. The corner of the resulting muscle flap is fastened to the skin of the upper thigh. Suitable lengths of vein and artery are cleared from the surrounding fat and connective tissues and the few small arterial branches ligated for 2 or 3 cms. from the point of canulation, which should be near the knee in order to leave free access to the vein for the injections.

Technic of assay.—After the blood pressure has reached a constant level the preparation is ready for the assay. It is convenient to adjust the speed of the drum to a rate of about 1 cm. in 5 minutes so that responses produced by injection at 3 to 5 minute intervals will be closely spaced and thus facilitate comparison. The injections are made into the crural vein with a 0.5 cc. or 1.0 cc. tuberculin syringe and a 1 cm. 27 gauge needle in order to minimize blood reflux. For this same reason succeeding injections are advisedly made into the same point of puncture. In suitable subjects injection may be made at intervals of 3 to 5 minutes. The standard pituitary solution (U. S. P. XI) is diluted 5 or 10 times with 0.9 per cent NaCl solution, the lesser dilution being preferable in cases of low blood pressures. With such dilutions a suitable response is obtained with 0.1 cc. $\pm$ 0.05 cc. In any case the dose should be so adjusted that the response is a fall of blood pressure of 20 to 40 mms., since that is the range of greatest sensitivity. In this range the response is almost directly proportional to the size of the dose. After the proper dose is determined, alternate injections or alternate

pairs of injections are made with the standard and unknown solutions until a dose of the unknown is found which will elicit the same fall of blood pressure as the standard. Fig. 1 is an example of the assay of two pituitary preparations. There appears to be no essential reason for the injections to be made at regular intervals. Fluctuations in

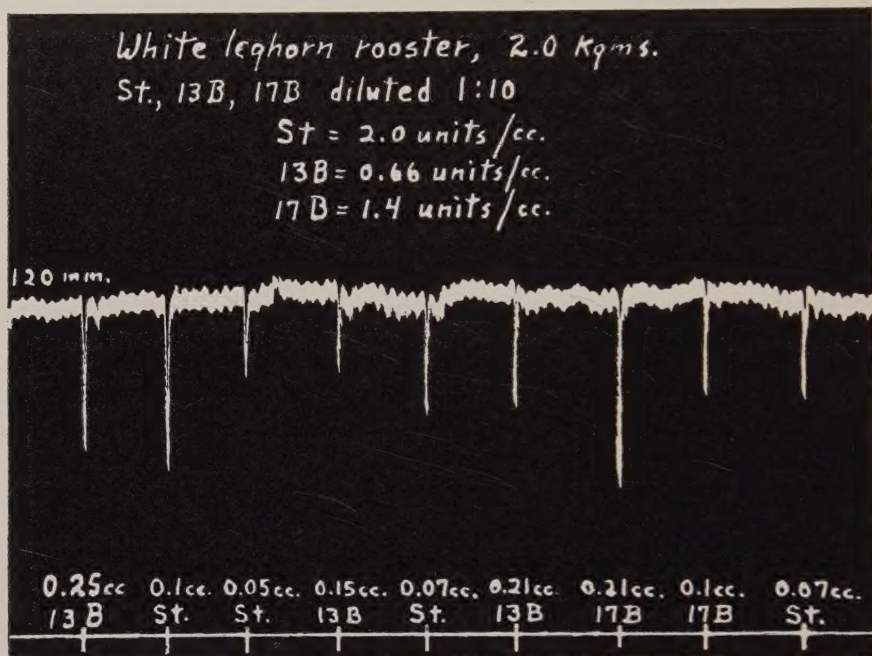


FIG. 1

Blood pressure tracing demonstrating the assay of two posterior pituitary extracts, 13B, and 17B, of unknown strength. Injections at 3 to 4 minute intervals.

blood pressure frequently interfere with such a sequence, and in these cases it is best to wait until the pressure has returned to the original level or has apparently established a new level before proceeding with the assay. HOGBEN (5) and MORASH and GIBBS (7) observed that the depressor response to pituitary preparations is frequently followed by a pressor-effect, especially after the injection of vasopressin (MORASH and GIBBS). In this laboratory the secondary rise was found to be a constant effect of the first small assay doses of pitressin or pituitary solutions in which the pressor-oxytocic ratio was greater than 4 or 5. This pressor effect interferes with a regularly spaced sequence of injections because of the prolonged return of the elevated blood pressure to normal.

There was some evidence that the rate of the injection influences to

a small degree the response to the posterior pituitary solutions. This might be expected in view of the fact that the response is exceedingly rapid and fleeting. The influence of this factor, however, may be obviated by keeping the dose at a volume which can be injected instantaneously. Volumes up to between 0.2 cc. and 0.3 cc. can be thus administered without introducing any significant error. After the slowly developed tolerance of the animal has reached a point where such volumes of the original dilutions do not give a satisfactory response more concentrated solutions should be used. It has been shown previously that the sensitivity to pituitary extract decreases with decreasing blood pressures. It is sometimes observed that under the conditions of the assay the basal blood pressure level undergoes a gradual decline throughout a period of several hours until it reaches as low as 90 to 100 mms. Hg. At this stage, the sensitivity is considerably diminished because of both the low pressure and the previous pituitary injections. Likewise, it is important to note, at this level of blood pressure a decrease as large as 30 or 40 mms. approaches a maximum response in the vicinity of which the assay preparation does not discriminate well between varying doses. Accordingly, the dose should be adjusted to one which will produce a fall of about 20 mms. or less. Occasionally when the blood pressure has subsided to undesirable levels large doses of ephedrine, 4 to 8 mgms. per kgm., have successfully established a long lasting elevation of pressure during which the sensitivity is enhanced and assays may be executed without interference from the effects of the ephedrine.

In the experience of this laboratory it has not been found that when a chicken is once prepared for assay purposes it is unsatisfactory for that purpose. The most frequent troublesome features are low blood pressure, fluctuations in blood pressure, and unusually rapid development of tolerance toward the oxytocic factor. None of these, singly or together, however, render the subject wholly unfit for the assay of pituitary extracts. As a rule, a chicken selected and prepared according to the specifications and procedures outlined above constitutes a stable subject for 6 to 12 hours, upon which more than a half dozen pituitary preparations may be satisfactorily assayed.

TOLERANCE OF THE CHICKEN TO PITUITARY

SCHAUMANN (15) reviews the literature dealing with the toxicity of pituitary extracts and pitressin for different animals. GEILING and CAMPBELL (16) working with the cat, and HARTMAN and GEILING (17)

with the monkey demonstrated a striking tolerance of the animals to large quantities of pituitary extracts administered intravenously. DI MATTEI (2) reported that the cock was very resistant to large doses of pituitary extract. Observations made in this laboratory also indicate a marked tolerance of the chicken. One hundred and seventy units of

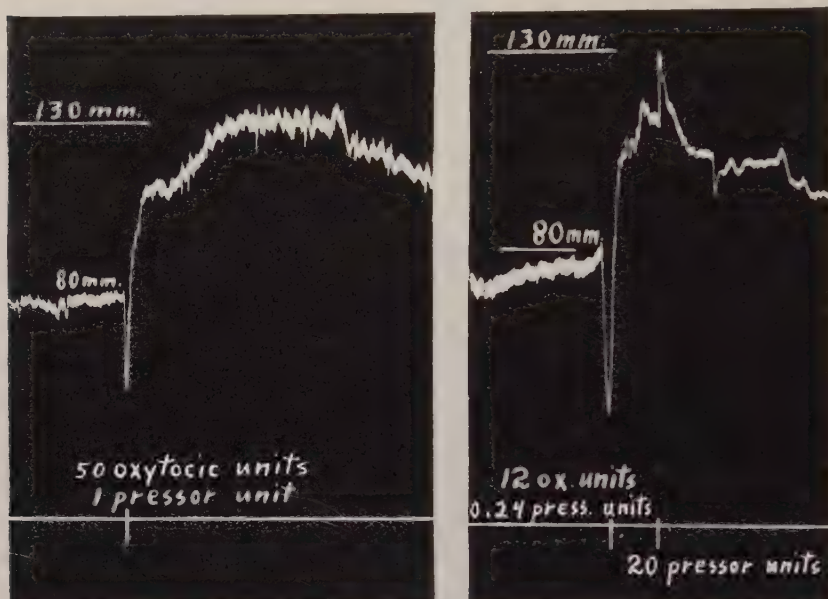


FIG. 2

Tracings showing secondary rise after large doses of pitocin, and pure rise with pitressin during complete immunity to the oxytocic substance.

pitressin given intramuscularly over a period of one hour had no effect on blood pressure, although it caused a rapid respiration and at first a paling of the comb followed soon by a persistent cyanosis. Fifty units of pitocin injected intravenously caused the typical transitory fall of blood pressure followed by the same changes in respiration and comb color as occurred after the large intramuscular doses of pitressin. It seems a remarkable fact that in a fresh chicken preparation 0.01 unit of pituitary will produce a blood pressure fall of about 20 mms., and a dose 5000 times as large will elicit a response which differs only in that it is greater and is followed by a definite pressor effect due to the large accompanying dose of pressor fraction (FIG. 2).

The rate of development of tolerance to the depressor effect of pituitary is primarily a function of the dose, although the length of the interval between injections is an important factor. Most previous

workers (3, 4, 6, 7, 8, 9) stated that a marked tolerance readily appears after succeeding doses, although the first few doses may give almost equal responses. In most cases, however, they gave large doses and allowed long intervals between. No observations seem to have been made of the effect of very small doses at short intervals of 3 or 4 minutes.

Since possible fluctuation in sensitivity of the subject is a factor to be considered seriously in any method of assay, some attention was directed towards the nature of the tolerance produced by repeated injections of pituitary solutions in the chicken. The results of many sequence of injections of different sizes have led to the following primary conclusions. 1. A dose of pitocin so large can be given that no subsequent injection of any size will produce the slightest change in blood pressure if given less than 15 to 20 minutes after the initial administration. The minimum dose which caused immediate absolute immunity was not determined, but the effect was observed after 25 units. 2. An amount so small can be given that no significant difference in response can be detected in any series of 3 or 4 (FIG. 3), and after 100 injections at 5 minute intervals the quantity required to give the original response

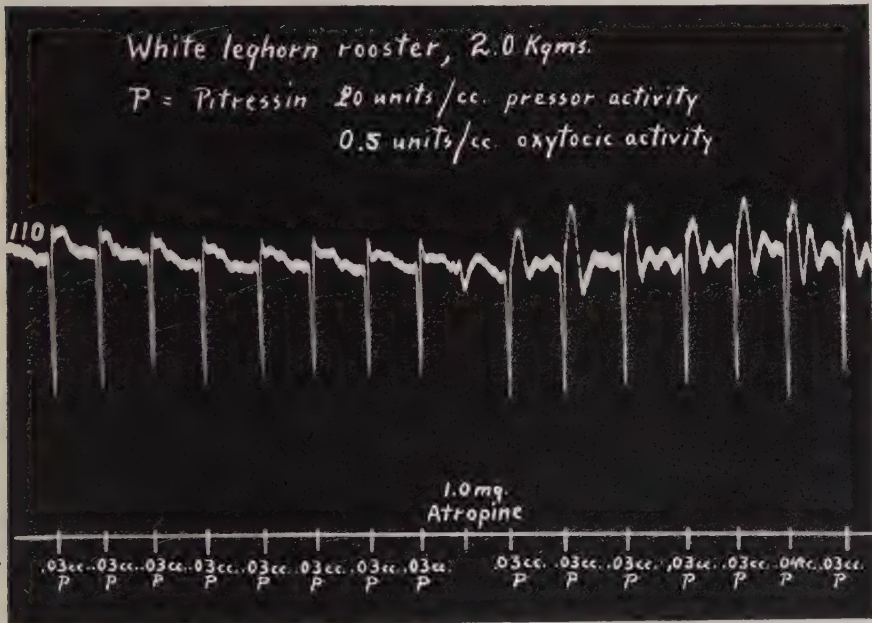


FIG. 3

Tracing showing effect of a series of equal injections of pitressin before and after atropine. Note persistence of fall, secondary rise, augmented fall and augmented secondary rise. 3 to 4 minute intervals.

is only 5 to 8 times the starting dose. A beginning injection of 0.01 to 0.03 unit is one of which will cause a partial tolerance to be developed slowly. The rate of onset of tolerance was augmented by increase in dose. A series of 4 doses of 1 unit each at 4 minute intervals gave a practically total immunity (FIG. 4). A series of 6 doses of 0.3 to 0.6

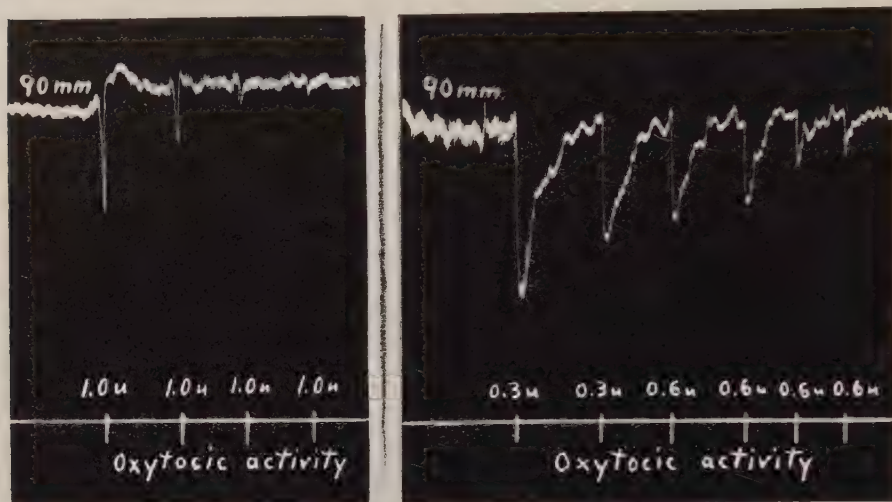


FIG. 4

Tracings comparing rate of development of tolerance with different doses. 2 to 4 minute intervals.

unit did not produce the same degree of tolerance. No recovery was seen to occur from the partial tolerance produced by many small injections. It was not observed that in a series of small assay doses any response was greater than a preceding one produced by the same amount, regardless of the length of the interposing interval. It might be thought that the anesthetic or slowly changing state of the animal could be responsible for the slowly developed tolerance observed to accompany small doses of pituitary. This development of tolerance, however, seems to cease when the injections are stopped, because if a 2 or 3 hour interval is allowed, the responses at the end of this period are equal to those before, no tolerance having been developed and no recovery having taken place in the meantime.

LIMITATION OF THE METHOD

Logically there arises the question of how results obtained by this method compare with those obtained by the accepted official method. It was first observed while assaying extracts of the posterior lobes of

whale hypophyses, prepared according to U.S.P. XI directions for pituitary solutions of unknown strength, that there was a discrepancy in results obtained by the guinea pig uterine method and the chicken depressor method. Some of the whale pituitary extracts assayed noticeably higher by the depressor method than by the uterine method. McClosky, Miller and LeMessurier (18), and Geiling (19) showed that the pressor-oxytocic ratio of certain whales is high, i.e., the pressor substance is present in an amount equal to that of the Standard Pituitary Powder, whereas the oxytocic substance exists in quantities only 10 to 40 per cent of the standard. The two methods gave different results also in assays of armadillo posterior pituitary extracts in which there is likewise a disproportion in the pressor and oxytocic components as shown by Oldham (20). The disagreement was not found, however, in the pituitary of the white whale in which there is no disproportion in pressor and oxytocic activity (advance communication). Thus it appeared that only those extracts in which the pressor component existed in materially larger amounts than the oxytocic component assayed higher by the depressor than by the uterine method. The possibility of the existence in the whale pituitary extracts of depressor bodies other than the oxytocic factor was excluded by treatment of such extracts with alkali and subsequently testing them for depressor effect in the chicken. The depressor activity completely disappeared under this treatment, proving there were no residual depressor substances. This evidence pointed to the pressor fraction of pituitary as the factor responsible for high oxytocic values obtained in the depressor assay of solutions with a high pressor-oxytocic ratio. It remained to be determined how much higher than the oxytocic content must the pressor content be in order to increase the assay. Table III contains results of assays of whale and armadillo pituitary extracts by the two methods. These data show that the oxytocic-pressor ratio must be less than 0.4 in order to give depressor assay values appreciably higher than those obtained by the uterine assay. Assuming that this interference was of a progressive nature and could only be detected after reaching a magnitude exceeding the limits of error of the method, it was at once suspected that if solutions with an oxytoci-pressor ratio less than $\frac{2}{5}$ assayed too high against a standard in which the ratio was 1, then solutions with an oxytocic-pressor ratio appreciably greater than $\frac{5}{2}$ might assay too low against the same standard. This suspicion was not confirmed, however, for assays of solutions of pitocin⁽¹⁾ gave similar results by both methods

(¹) The author wishes to acknowledge indebtedness to Parke, Davis and Company for generous supplies of pitressin and pitocin.

(TABLE III). This might be interpreted as meaning that the pressor fraction does not augment the depressor action of the oxytocic fraction unless it is present in quantities definitely larger than the oxytocic fraction. In view of this interpretation then, the above suspicion was unwarranted, for one would not expect the pressor fraction of the standard solution to exert augmentory effect on the depressor action if it is true that almost three times as much pressor as oxytocic activity must be present to augment the depressor action of the oxytocic substance.

TABLE III

Comparison of some results obtained by the Uterine and Chicken Depressor Methods of Assay

Pituitary Solutions	Uterine Method Units per cc.	Depressor Method Units per cc.	Per cent Error	Pressor Activity Units per cc.	O/P	O'-O
						O
Sperm whale	0.2	0.5	150	2.0	0.1	1.5
Armadillo	0.2	0.6	200	2.0	0.1	2.0
Blue whale	0.8	0.8	0	2.0	0.4	0
White whale A.	2.0	2.0	0	2.0		
White whale B	1.6	1.4	0	1.6		
Commercial pitocin.	10	10	0	—		
Purified oxytocic fraction A	48	46	0	—		
Purified oxytocic fraction B	25	25	0	—		
Purified pressor fraction R ₄	1.6	8	400	40	0.04	4.0
Purified pressor fraction 910	1.5	5	230	25	0.06	2.3
Pitocin-pitressin mixture I.	8	8	0	20	0.4	0
Pitocin-pitressin mixture II	5	7	40	22	0.23	0.4

To confirm the conclusion drawn from data obtained in assays of the whale and armadillo pituitary extracts pitressin and mixtures of pitressin and pitocin in different proportions were assayed for oxytocic activity by both methods. The results of these experiments (TABLE III) are wholly confirmatory, and prove that the pressor component must exceed the oxytocic component by more than 250 per cent before it will introduce significant discrepancy in assay results by the two methods. It may also be seen that the degree of discrepancy increases as the oxytocic-pressor ratio decreases. Fig. 5 demonstrates this relation. The oxytocic-pressor ratio is plotted on the abscissa. On the ordinate is plotted the ratio of the unit excess of the depressor assay over the uterine assay

to the value of the uterine assay. If O represents the official oxytocic assay value in units per cc. found by the uterine method, and P represents the pressor assay value in units per cc., then O/P is the oxytocic-pressor ratio. If O' is the oxytocic assay value as found by the depressor method then $\frac{O'-O}{O}$ is the ratio of excess oxytocic assay to official oxytocic assay.

The preceding discussion admits of the conclusion that the pressor

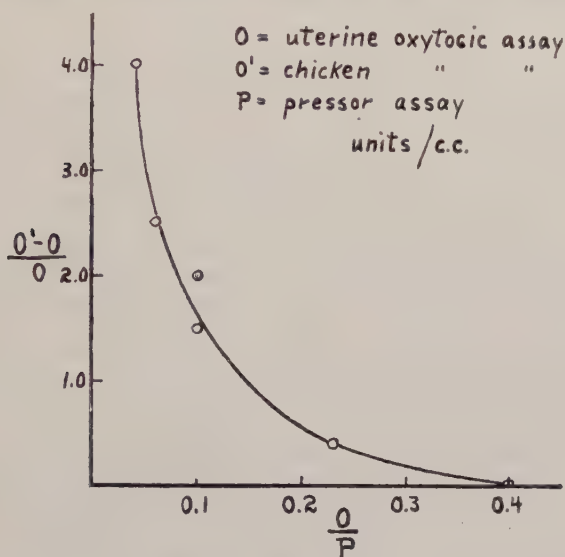


FIG. 5

Curve expressing the relation between degree of difference in oxytocic and pressor content of a solution and degree of assay error as compared to official uterine method.

substance of posterior pituitary, when present in relatively large amounts, interferes with the chicken depressor assay of oxytocic activity to give high results. In reaching this conclusion data obtained from uterine assays have been used. However, there is no positive proof that the pressor substance when present in relatively large amounts does not interfere with the uterine assay of oxytocic activity to give low results.

Assuming the depressor method to be the one giving misleading results, the possible mechanism of the augmentation of the depressor response by the pressor hormone has received some consideration, and experiments have been carried out to determine the physiological basis of the effect.

A priori it would be expected that pitressin, which is a vaso-constrictor in birds [HOGBEN (5), MORASH and GIBBS (7)] as well as in mammals,

would tend to cut short the fall in blood pressure caused by pitocin and thereby reduce the oxytocic assay value. It has been amply demonstrated that this does not occur, but that, on the contrary pitressin apparently augments the depression caused by the oxytocic substance. The possible causes for this phenomenon are as follows: (1) a generalized, regional or specialized vasodilator effect of the pressor factor, peripheral or central in origin due to an action similar to or different from that produced by pitocin; (2) a deleterious action of pitressin on the heart of the chicken under the conditions of the assay.

Direct experimental evidence bearing upon the first possibility is exceedingly difficult to derive for a number of reasons. Pitressin or pitocin totally free of significant amounts of the other are not yet available. A dose of pitressin large enough to produce an effect contains sufficient oxytocic impurity to render interpretations of organ volume studies difficult and unreliable. If the pressor substance does cause a primary vasodilation, it is so slight and fleeting that it probably would not be manifested as a measurable change in organ volume. An injection of pitressin after a large dose of pitocin, as shown by MORASH and GIBBS (7) and confirmed in this laboratory, causes a pure rise of blood pressure. This does not prove that the pressor factor does not possess some depressor activity because a dose of pitocin large enough to effect complete immunity to the oxytocic principle, so that the effect of pitressin alone might be studied, contains considerable pressor impurity. This is very likely sufficient to produce at least a partial tolerance towards the pressor fraction also. MORASH and GIBBS (7) pointed out that a tolerance is more readily established towards pitressin than towards pitocin. Apparently then the depressor action of the pressor substance, if it exists, disappears before the pressor effect in the process of desensitization.

A central depressor action of the pressor hormone is considered quite improbable in view of the fact that the augmented depressor effect occurs equally in unanesthetized, lightly anesthetized, or deeply anesthetized chickens. Studies of the reaction to pitressin and pitocin of isolated segments of arteries and veins of different organs may possibly throw some light upon the problem, although the impure character of these drugs would still be a complicating factor. A number of investigators (8, 21, 22, 23) have observed a fall in portal pressure in cats and dogs upon intravenous administration of vasopressin. In cats they attributed this to an arterial and capillary constriction which diminished the flow into the portal system. HOLTZ (8) described the effect in dogs, where it occurred in spite of increased blood flow to the splanchnic area, as being

mainly due to to dilation of the hepatic veins. He gave evidence that the oxytocic fraction was responsible for this effect and inferred an action analogous to that of pitocin in the fowl. According to HOLTZ, the mesenteric veins of dogs and cats were slightly dilated by vasopressin. The possibility of dilation of blood vessels due to the pressor substance gives an approach to the question of the cause of the depressor effect in birds. If this occurs in the fowl, it may be of general or local importance. The first change seen in the comb after an injection of a sufficient amount of pituitrin, pitressin, or pitocin, is a distinct paling, as described by MORASH and GIBBS (7), followed by a cyanosis with excessive doses. The fall of blood pressure in the chicken is so evanescent that no parallel change in comb color can be detected. The onset of pallor in the comb is always after recovery from the fall of blood pressure. A long series of small assay doses produces no detectable changes in the circulation of the comb. The pallor established by injection of large doses of pitressin is not affected by a maximum fall of blood pressure, as may be produced by pitocin. SACHS (24) observed the occurrence of pallor in the skin of man in spite of no change or even a fall in blood pressure. He interpreted this to mean that the capillaries and venules contracted actively with pituitary extract and were not emptied merely by contraction of the arterioles. In the rooster's comb the interpretation is that capillaries and veins are actively contracted and are not filled by a dilation of arterioles.

EFFECT OF PITRESSIN AND PITOCIN ON THE ISOLATED HEART

PATON and WATSON (3), working with the duck heart in situ, observed an increase in ventricular amplitude with no change in rate following intravenous injection of pituitrin equivalent to several units. This occurred with vagi intact or cut, but complete atropinization abolished the effect upon amplitude. These workers claimed that this cardiac response to pituitary was not secondary to alterations in blood pressure because it occurred in cases where the pressure underwent no significant change. They concluded that pituitrin acts directly on the ventricular muscle and not on the augmentor mechanism, since, as they state, the augmentor nerves have no influence on the ventricles. MORASH and GIBBS (7) using the fowl attempted to repeat the experiments of PATON and WATSON. Their results were not striking and led them to no conclusions regarding the cardiac effect of pituitary in the bird. They emphasized the difficulty of interpreting changes in amplitude of the

intact heart, subject as it is to peripheral changes. DIETEL (9) obtained an increase in heart activity of the bird with orasthin. He did not state the conditions of the experiment or the dose used.

Experiments were carried out in this laboratory to test the effect of pitressin and pitocin on the isolated chicken heart perfused with oxygenated Ringer-Locke's solution at a pressure of 750 mms. perfusing fluid and a temperature of 42° (normal chicken temperature 41.5°). Observations were taken of the rate of coronary flow and rate and amplitude of ventricular and auricular contractions. The results of these experiments showed that pitocin increases the coronary flow and the amplitude of both auricles and ventricles without change in rate. PATON and WATSON (3) observed no effect upon the auricles of the duck. Pitressin produced the same effect, but such large doses were required that the action was attributed to the oxytocic impurity. The coronary flow was augmented to a detectable degree by the injection of 0.25 unit of pitocin. The response could be obtained repeatedly, but an immunity was slowly developed. Five units of pitocin were required to elicit a definite augmentation of heart action. These doses necessary to affect the isolated heart are much larger than those used for assay purposes, where 0.01 unit produces a marked fall of blood pressure. Such a small quantity injected into the intact chicken causes a slight increase in rate and amplitude of the heart, an effect which is constant whether produced by 0.01 unit as pitocin or as oxytocic impurity in pitressin. These changes must be secondary to the fall in blood pressure for they occur with doses which must be multiplied 50 times to influence the activity of the isolated heart. If there were a central stimulation through the augmentor mechanism it would have to be mediated through the auricles, for PATON and WATSON (3) claim that the augmentor nerves have no effect on the ventricles.

The question arose whether the anesthetic used could render the heart susceptible to a toxic action of the pressor substance. RAGINSKY and STEHLE (25) working with the heart-lung preparation of the dog reported that the cardiac depression caused by pituitary extract is exaggerated 20 times by the presence of sodium-phenobarbital. The explanation they suggested for this was that the barbiturate reduced the work capacity of the heart to such an extent that the coronary constriction produced by the pituitary exerted a more harmful effect. This explanation is borne out in experiments on the chicken heart perfused with Ringer-Locke's solution containing 50 mgms. of sodium-phenobarbital in 100 ccs., double the concentration used by RAGINSKY and STEHLE (25). During perfusion with this medium the cardiac

amplitude was about half as high as during normal perfusion, and coronary flow was more rapid. As much as 12 units of pitressin had no effect on such a depressed heart, and pitocin produced slight stimulation of amplitude and coronary flow. This resistance to pitressin in the presence of phenobarbital was anticipated, since the pressor factor does not cause a coronary constriction in the chicken heart as it does in the dog. These experiments lead to the conclusion that the pressor fraction does not in any way act on the heart of the chicken under phenobarbital to augment the fall of blood pressure produced by the oxytocic fraction. The possibility of central depression by stimulation of the inhibitory mechanism is ruled out by the fact that atropine does not abolish the apparent pressor augmentation of the oxytocic depression.

Attempts to treat the assay preparation by various means in order to render it immune to a possible depressor action of the pressor hormone were unsuccessful. The logical procedure appeared to be the administration of a large amount of pitressin in order to establish a tolerance to its effect. However, if this was given intravenously, enough oxytocic substance accompanied it as impurity to produce partial tolerance to it also. If large amounts of pitressin were given intramuscularly the desired immunity did not develop. Numerous other attempts to abolish the undesirable action of the pressor principle failed. It has already been stated that atropine or vagotomy did not influence this effect. However, atropine did have an influence upon the vascular reaction to pituitary extracts. PATON and WATSON (3) reported that the fall of blood pressure in the duck was more prolonged after atropinization due to the abolition of the cardiac stimulation by pituitary extract. In this laboratory 1 mgm. of atropine injected intravenously was seen not only to prolong but also to augment both the depressor action of the oxytocic substance and the pressor action (FIG. 3), which appears secondarily after sufficient doses of pressor substance. LARSON (26) observed that vagotomy and destruction of the carotid sinus nerves increase the pressor response of dogs to pituitary extract, proving that these mechanisms normally inhibit this response. The same mechanisms would be expected to operate in the bird to suppress both the fall and the rise in blood pressure due to pituitary extracts. Evidence that this is true is seen in the fact that atropine or vagotomy augments both of these actions.

Observations upon the occurrence of the secondary pressor effect of pituitary in the chicken have led to the conclusion that a sufficient amount of the pressor substance will elicit this effect regardless of the accompanying oxytocic fraction. It had been observed (5, 7, 19) that

the fall in blood pressure in response to solutions with a high pressor-oxytocic ratio was regularly followed by a secondary rise. Fig. 3 shows that doses of 0.6 unit of pressor activity in the presence of 0.15 unit of oxytocic activity give a consistent though not a marked secondary pressor effect when the blood pressure is fairly high. When the pressure is low the sensitivity to the pressor action is greater. A dose of 0.2 to 0.3 unit of pressor substance in the presence of 12 units of oxytocic activity on several occasions gave a marked rise with the blood pressure as low as 80 mms. Hg (FIG. 2). It would appear then that the secondary pressor rise depends on the absolute amount of pressor substance injected and on the height of blood pressure rather than on the pressor-oxytocic ratio, and that this effect in the chicken is not masked by the fall in blood pressure. This is logical since the pressor effect is quite sustained and is manifested after the transitory depressor effect wears off.

DISCUSSION

The advantages of the method described for the bioassay of pituitary extracts are evident when one considers the simplicity of the technic and equipment required. A minimum of experience in blood pressure work is necessary before the assayist can without difficulty on the same chicken in one day assay a half dozen pituitary preparations, each with as great a degree of accuracy as can be expected in biological assay. The assay subject is moderately sensitive, but not so sensitive as to render it susceptible to interference by traces of extraneous materials. This is the distinct disadvantage of the guinea pig uterus. The sensitivity does not fluctuate like that of the uterus, but decreases slowly and steadily in a predictable manner. The chickens are easily available, conveniently kept under laboratory conditions, easy to work with, and, if properly selected, they are 100 per cent good for assay purposes. The limitation of the method is a rather narrow one. Assay values agreeing with those obtained by the official method can be obtained with pitocin and whole posterior pituitary extracts. Many experiments were executed and attempts at correction made with the assumption that the presence of a large proportion of pressor principle in a solution increases the depressor effect in the fowl. As stated this assumption was based wholly upon a faith of two decades that the activity of the guinea pig uterus was purely a function of the oxytocic component and totally independent of the pressor fraction. The only evidence of pressor augmentation of the depressor response in the bird is the fact

that this apparent augmentation appears to be partly diminished when large amounts of pressor hormone are administered in the attempt to establish a tolerance towards its action. Mention has previously been made of the absence of proof that the pressor hormone does not interfere in the official method to give low oxytocic assay values of solutions in which the pressor factor predominates. Pitocin has been observed to exert an action on smooth muscle antagonistic to that of pitressin. GRUBER and KOUNTZ (27) observed that pitocin dilated the coronary arteries of the perfused isolated rabbit heart. This is directly opposed to the action of pitressin, which, all previous workers agree, constricts the coronaries of the rabbit heart. Furthermore, the oxytocic fraction dilates and the pressor fraction constricts the arterioles of birds. MELVILLE (28), studying the comparative effects of pitressin and pitocin on blood pressure and intestinal activity in the unanesthetized dog, discovered that a given dose of pressor substance, as determined by pressor assay, produced a much smaller response when administered as pitocin than as pitressin. He concluded that the oxytocic constituent, in sufficient doses, exerts an antagonistic influence upon the action of the pressor component on blood pressure and intestinal activity. Thus, with several reported examples of antagonism between pressor and oxytocic principles and no example of an additive effect of these two factors in experimental animals, except the questionable one described in this paper, the suspicion at once arises that the pressor component when present in preponderant amounts inhibits the uterine action of the oxytocic constituent enough to account for the discrepancy in results obtained by the two assay methods. Until this question is settled the avine depressor oxytocic assay technic should be of value as a preliminary and a check method in official circles, and in research laboratories as a quick and reliable method for the assay of the oxytocic activity of pitocin and pituitary extracts.

SUMMARY

1. A simple technic is described for the bioassay of posterior pituitary extracts employing the depressor response of the domestic fowl as the physiological basis.
2. Data are presented to show that the white leghorn rooster, 1.8 to 2.2 kgms. in weight, and anesthetized with 200 mgms. per kgm. of sodium-phenobarbital, is the most satisfactory assay subject.
3. The tolerance of the chicken towards pituitary is shown to be

high and the responses to repeated small doses to be so consistent as to permit several tests on the same assay preparation.

4. Comparative assays by this and by the official guinea pig uterine method reveal the fact that only when the pressor-oxytocic ratio of a pituitary solution is above 2.5 is the oxytocic assay value obtained by the depressor method higher than that obtained by the official method. In all other cases results by the two methods agree.

5. Possible causes for the apparent augmentation of the oxytocic depression by the pressor component are considered.

6. Experiments on the perfused isolated chicken heart prove that the phenomenon is not the result of action of the pressor substance or the anesthetic on the myocardium or coronary arteries.

7. Pitocin is found to increase coronary flow and stimulate the activity of the heart. Pitressin has no effect not attributable to its oxytocic impurity.

8. Atropinization augments both the fall and the secondary rise in blood pressure produced by the injection of pituitary extracts.

9. The extent of the secondary pressor effect is dependent upon the beginning blood pressure level and upon the absolute dose of pressor substance and is not influenced by the accompanying dose of oxytocic substance.

10. The advantages of the chicken depressor method of assay are outlined.

11. The validity of the official guinea pig uterus method in the assay of pituitary solutions with high pressor-oxytocic ratio is questioned.

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